REMARKS

Claims 2, 5, 7-11, 13-15, 18, 20, 22, 24, 28, 29, 31, 33, 38, 40, 41 and 43 are pending. Applicants have amended claims 9, 22, 31, 38, and 43 to recite "wherein the autologous bone marrow-mononuclear cells are isolated from bone marrow"; support for these amendments may be found in the specification as filed *inter alia* at page 8, line 19 to page 9, line 9. In particular, the Applicant wishes to direct the Examiner's attention to the specification as filed, at page 8, lines 24-26, where it is disclosed that the autologous bone marrow cells are pelleted and then resuspended in medium. The specification then discloses that "Low density bone marrow mononuclear cells are separated from the suspension."(See, page 8, lines 26-28). Since the suspension contains the autologous bone marrow cells, this disclosure is a description of the recitation in the presently amended claims that "autologous bone marrow-mononuclear cells are isolated from bone marrow."

No new matter is presented by this Amendment. Accordingly, claims 2, 5, 7, 9-11, 13-15, 18, 20, 22, 24, 28-29, 31, 33, 37-38, 40-41 and 43 will be pending. Reconsideration and allowance of this application is respectfully requested.

Rejections under 35 U.S.C. § 103(a)

Kobayashi et al.

Claims 2, 5, 7, 9-11, 13-15, 18, 20, 22, 24, 28-29, 31, 33, 38, 40-41 and 43 have been rejected as being unpatentable over Kobayashi et al. (J. Surgical Res. 2000 Apr; 89(2):189-95).

The Examiner states that Kobayashi et al. teach a method of administering to a mammal autologous bone marrow (BM) cells for the enhancement of angiogenesis in an in vivo heart model and that such administration to artificially created ischemic regions of the heart caused elevated angiogenesis in these ischemic regions. The Examiner points to Kobayashi et al.'s introduction, which states that "bone marrow contains many kinds of immature cells which could differentiate into hematopoietic cells and endothelial progenitor cells (*See,* Kobayashi et al. p. 189, Col. 2). The Examiner asserts that "Although not characterized as having administered mononuclear cells (MNC), the BM cells administered by Kobayashi et al. would also contain MNCs. (Office Action, page 4, lines 16-18).

The Examiner asserts that it would have been *prima facie* obvious to treat ischemic

tissue by administering BM-MNCs from a human to generate the formation of capillaries or collateral vessels through angiogenesis. The Examiner further states that one of skill would have been motivated to do so because the administration of BM cells by Kobayashi et al. resulted in the revascularization of the ischemic region through angiogenesis in a rat ischemic heart model. The Examiner asserts that BM cells administered by Kobayashi et al. "achieved the same result of administering the BM-MNCs as that instantly claimed, and because the BM cells also contain BM-MNCs, one of skill would have a reasonable expectation that BM (sic) when administered would result in the same collateral and capillary formation". The Examiner notes that the claims recite isolated BM-MNCs, however, no specific recitation or limitation which refers to the purity of the isolation. The Examiner concludes that one of skill would have found reasonable motivation to do so in humans because the administration of BM to ischemic regions in the in vivo model taught by Kobayashi et al. was successful in revascularizing the damaged heart tissue, and one of skill would have reasonable expectation that such a model is predictive of human success.

Applicants respectfully traverse the Examiner's rejection and maintain that presently pending claims 2, 5, 7, 9-11, 13-15, 18, 20, 22, 24, 28-29, 31, 33, 38, 40-41 and 43 are not obvious over Kobayashi et al.

In order to establish a *prima facie* case of obviousness, three criteria must be met: First there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art references (or references when combined) must teach all the claim limitations. (See, MPEP 2143) The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The present invention is directed to methods of forming new blood vessels in cardiac muscle tissue in a subject, wherein the subject is a human, methods of increasing blood flow to cardiac muscle tissue in a subject, wherein the subject is a human, method of treating diseased cardiac muscle tissue in a subject, wherein the subject is a human, methods of increasing angiogenesis in diseased cardiac muscle tissue in a subject, wherein the subject is a human and methods of treating heart failure in a mammal, wherein the subject is a human, which methods comprises: a) isolating autologous bone marrow-mononuclear cells from the human, wherein the autologous bone marrow-mononuclear cells are isolated from bone

marrow; and b) transplanting locally into the cardiac muscle tissue an effective amount of the autologous bone-marrow mononuclear cells, resulting in formation of new blood vessels in the cardiac muscle tissue. (Emphasis added) Therefore, the claims specifically recite BM-MNCs which have been isolated, *i.e.*, removed from bone marrow cells which contain various cell types, as well as other components. Applicants note that the techniques for the isolation of MNCs from bone marrow are well known by one of skill in the art. Since one of skill in the art can readily isolate MNCs from bone marrow with a known degree of purity of MNCs, no specific recitation or limitation which refers to the purity of the isolation of MNCs is required.

Initially, Applicants respectfully direct the examiner's attention to the abstract of Kobayashi et al, which concludes by specifically stating that "bone marrow implantation could be a novel and simple method to induce therapeutic angiogenesis". No suggestion or teaching is provided with respect to any particular cellular component or growth factor which may be found among the various components of bone marrow cells.

Kobayashi et al. discloses collecting bone marrow from rat femur and tibia, placing the bone marrow into phosphate buffered saline (PBS), forming simple bone marrow cells suspensions by gently pressing bone marrow segments through a fine wire mesh, removing red blood cells from the suspensions and suspending the bone marrow cells in PBS (*See,* Kobayashi et al. p. 190, *Preparation of rat bone marrow cells*). Kobayashi et al. resuspended the aforementioned bone marrow cells in PBS containing 20 mmol/liter of the dye CFSE and incubated the suspension for 10 minutes at 37° with gentle mixing. After three washes with PBS, the labeled bone marrow cells were injected into the ischemic heart (*See,* Kobayashi et al. p. 190, *Monitoring of the implanted bone marrow cells in the infarcted myocardium*).

Therefore Kobayashi et al. do not ***isolate*** autologous mononuclear cells ***from the bone marrow*** of a human, and transplant these specific cells, as required by the claimed methods. Accordingly, Kobayashi et al. does not teach all the claim limitations of applicants invention.

Although Kobayashi et al. notes that bone marrow contains many kinds of immature cells which could differentiate into hematopoietic cells and endothelial progenitor cells, Kobayashi et al. does not teach or suggest that any such immature cells be removed from the bone marrow's mixture of various cells. Indeed, Kobayashi et al. does not suggest that any one particular cell type be isolated from the whole bone marrow cells and be transplanted locally into the cardiac muscle tissue. Neither is there any teaching or suggestion that mononuclear cells, specifically, be removed from the bone marrow for such transplantation. Applicants respectfully direct the Examiner's attention to Kobayashi et al.'s discussion of the significantly

increased protein expression of inflammatory cytokines in the BMI [bone marrow implanted] group, wherein Kobayashi et al. state that the main potential source for these inflammatory cytokines are monocytes, macrophages, and granulocytes, all of which the bone marrow contains. (*See,* Kobayashi et al. p. 194, Col. 1, first paragraph). Kobayashi then suggests that the angiogenesis observed depends on those inflammatory cytokines (See p. 194, Col. 1, lines 26-29) : "It is suggested that angiogenesis induced by the BMI treatment in this model is related to inflammatory cytokines ..." This is a clear teaching away from the presently claimed invention, which requires isolation of a single cell type, because Kobayashi is suggesting that it is important to include the monocytes, macrophages, and granulocytes because those cell types are the cells that produce the inflammatory cytokines upon which angiogenesis depends.

However, Kobayashi does not teach or suggest that any of these cells be isolated from the bone marrow cells containing all of them. Therefore, contrary to the Examiner's assertion, one of skill would not have any motivation to ***isolate MNCs from bone marrow*** based on Kobayashi et al.'s teaching because only the administration BM cells, not MNCs isolated therefrom, is taught by Kobayashi et al. for induction of angiogenesis. Therefore, one of skill in the art would have a reasonable expectation that implantation of BM, not the isolated MNC fraction thereof, would induce angiogenesis. The Examiner has not shown any nexus between Kobayashi's results, obtained with BM cells from which only red blood cells have been removed, to applicants' administration of MNCs, as claimed, to provide a reasonable expectation of success using MNCs isolated from BM.

Further, Kobayashi et al. speculate that "possibly, unknown growth factors might account for the angiogenesis induced by transplantation of bone marrow" and state that the contribution of implanted bone marrow cells to endothelial cells was not examined, but that further studies are necessary (*See,* Kobayashi et al. p. 194, Col. 1, first paragraph). Therefore Kobayashi et al. suggest that other factors besides the mixture of cellular components of bone marrow may be involved in angiogenesis. There is no suggestion to remove any cellular subfraction from the bone marrow cells. On the contrary, the presence of unknown factors, as described by Kobayashi et al. would suggest to one of skill that the entire fraction of bone marrow cells is required for administration so as to retain the unknown factors.

Finally, Kobayashi et al. state: "Further investigations on BMI treatment are required to clarify the optimal populations of whole bone marrow cells that will have the most angiogenic potency and to determine whether BMI will induce stronger angiogenesis in an ischemic environment." (*See*, Kobayashi et al. p. 194, Col. 2, first paragraph). Thus, Kobayashi et al.

suggest that populations of bone marrow cells, ***not any one specific cell type or mononuclear cells, in particular***, be used for further studies to ascertain optimal populations. Therefore, Kobayashi et al. provide no reasonable expectation of success of administration of MNCs isolated from the bone marrow used by Kobayashi et al.

Since Kobayashi et al. has not met the three criteria to establish a *prima facie* case of obviousness, Kobayashi et al. cannot render obvious the presently pending claims.

Accordingly, applicants respectfully request that the Examiner reconsider and withdraw the rejection under 35 U.S.C. § 103(a).

In view of the foregoing amendments and remarks, it is firmly believed that the subject invention is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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